Comparison of the application parameters of coccidia vaccines by gel and spray

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ABSTRACT Coccidiosis is an economically significant enteric disease caused by *Eimeria* species. Control of the disease is achieved through various means, including chemical anticoccidial drugs, ionophore antibiotics, and vaccination. Differences between the vaccines include the number of oocysts per dose (varying by as much as tenfold between vaccines), attenuation status of the oocysts, and the species present within the vaccine. Coccidia vaccines are typically administered via spray cabinet to day old chicks; however, a new gel-based delivery system that claims to elongate preening time and increase oocyst ingestion has been introduced and is specifically recommended for certain low dose vaccines. The purpose of this trial was to compare the application properties between high and low oocyst dose vaccines administered via gel and spray delivery systems to determine if application systems could potentially affect application success. The vaccines were mixed into gel and spray diluents per manufacturer's instructions, and samples were taken to assess how well the oocysts remained in suspension. Gel and spray application patterns were assessed by measuring the size and number of droplets applied onto a plexiglass sheet in a chick basket. Different size droplets were collected and oocyst enumeration and speciation were performed. Results show that no settling occurred after mixing in either diluent. As expected, the number of oocysts per droplet increased as droplet size of the spray administration increased but stayed constant in the uniform droplet size of gel administration. There was also a consistent number of oocysts found in each of the sections across the plexiglass sheet. Taken together, these data will aid poultry producers in deciding which delivery system will provide the best application in their production system.

Key words: coccidia vaccination, broiler, diluent

2018 Poultry Science 0:1–8
http://dx.doi.org/10.3382/ps/pey364

INTRODUCTION

Coccidiosis is an important intestinal disease in chickens caused by apicomplexan protozoa in the genus *Eimeria*. Multiple *Eimeria* species infect the chicken, the most significant in the broiler industry being *E. maxima, E. tenella,* and *E. acervulina.* Clinical infection with these species causes decreased weight gain, increased feed conversion ratio, mortality, and predisposition to secondary infections (Johnson and Reid, 1970; Reid and Johnson, 1970; Allen and Fetterer, 2002; Conway and McKenzie, 2007). Combined, these factors make coccidiosis a disease with significant economic impact. More than 60 billion chickens are produced worldwide each year, and the total global impact of coccidiosis is estimated to be in excess of $3 billion per year (Dalloul and Lillehoj, 2006; Blake and Tomley, 2014). Approximately, 80% of these losses are associated with the subclinical loss in performance parameters, including decreased weight gain and increased feed conversion, and the remaining 20% of costs include the cost of prophylaxis and treatment measures (Williams, 1999). In addition, coccidiosis is associated with increased intestinal colonization of *Clostridium perfringens* and *Salmonella enterica* serovars Typhimurium and Enteriditis, leading to further economic losses (Baba et al., 1982; Qin et al., 1996; Collier et al., 2008).

Historically, coccidiosis has been controlled using chemicals and ionophore antibiotics, but there is increasing use of live coccidia vaccines due to consumer preferences, loss of sensitivity to anticoccidial drugs, and governmental regulations (Jeffers, 1976; Vermeulen et al., 2001; VFD, 2015). These vaccines contain sporulated oocysts of different species, with *E. maxima, E. tenella*, and *E. acervulina* present in all vaccines. The first commercially available coccidia vaccine in the United States was introduced in 1952 (Edgar et al., 1952), with many other vaccines introduced since then (Lee, 1987; Shirley, 1989). These vaccines vary based on the number of total oocysts present in each dose of vaccine, the attenuation status of the organisms present,

Received June 4, 2018.
Accepted October 12, 2018.
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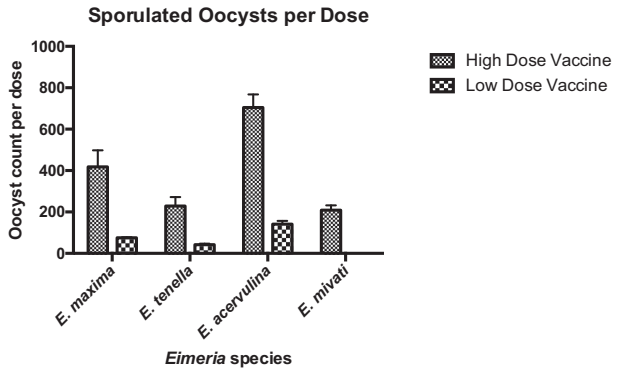

Figure 1. Number of oocysts present per 1,000 doses in high and low oocyst dose vaccines.

and the number and type of species present (Vermeulen, Schaap and Schetters, 2001; Chapman et al., 2002; Dalloul and Lillehoj, 2005). The total number of oocysts in a vaccine varies tremendously and ranges from less than 200 to approximately 3,000 oocysts per dose (Price et al., 2016).

Many different application methods have been used to apply the live oocyst vaccines, including administration on the farm through the drinking water, spraying on feed, use of gel droplets applied to the feed, and administration at the hatchery using ocular vaccination, gel bead delivery, and spray cabinets (Chapman, 1996; Chapman and Cherry, 1997; Danforth et al., 1997; Dasgupta and Lee, 2000; Chapman et al., 2002; Jenkins et al., 2012, 2013; Awad et al., 2013). In the United States, coccidia vaccines are most commonly administered through a spray cabinet to day old chicks at the hatchery. There is increasing interest for use of a gel diluent as studies have implicated that vaccination utilizing a water spray delivery system can result in uneven vaccine application resulting in chicks that do not receive any vaccine and chicks that ingest fewer or more oocysts than others (Chapman et al., 2002; Price et al., 2014). The chicks that do not receive vaccine in the hatchery will then be exposed to uncontrolled amounts of oocysts in the environment. These oocysts may be field derived or vaccine derived, as other chicks shed oocysts, resulting in worsened clinical signs in those birds.

Previous studies examining the effectiveness of different application methods of coccidia vaccines compared oocyst excretion and protection from challenge from gel-based, oral gavage, and spray vaccinated broilers (Dasgupta and Lee, 2000; Jenkins et al., 2012; Albanese et al., 2018) and have reached different conclusions about level of protection. Some studies found that gel administration elicited the greatest protection against an *E. maxima* challenge, while other studies found no difference in protection but differences in number of oocysts shed between different delivery methods (Danforth, 1998; Jenkins et al., 2013; Albanese et al., 2018). This study was performed to determine if the diluent used with the vaccine and method of vaccine delivery greatly influenced the number of oocysts applied in either a high or low dose vaccine.

MATERIALS AND METHODS

Vaccines

Two vaccines were used for these studies: a high oocyst dose vaccine, Coccivac-B52 from Merck Animal Health, and a low oocyst dose vaccine, Immucox III from CEVA Animal Health. As per the manufacturer's labels, Coccivac-B52 contains *E. acervulina*, *E. maxima*, *E. mivati*, and *E. tenella;* Immucox III contains *E. acervulina*, *E. maxima*, and *E. tenella.*

Oocyst Enumeration

Oocysts were enumerated for all parts of the trial utilizing a McMaster's chamber. Vaccine was mixed with an appropriate dilution of saturated salt water based on the concentration of oocysts. The resulting sample was then mixed and pipetted into a McMaster's chamber. The chamber was allowed to sit for 3 min so oocysts could rise to the top of the chamber, then were counted using the method of Conway and McKenzie. Oocysts were speciated according to the morphological characteristics of the different species present in the vaccine according to the manufacturer, including size and shape (Conway and McKenzie, 2007).

Sporulation Rate

To assess for sporulation, a sample was taken directly from each vaccine vial and diluted to an appropriate dilution for counting using a McMaster's chamber (the high oocyst dose vaccine was diluted 1:1000; the low oocyst dose vaccine was diluted 1:10). Based on the presence or absence of sporocysts and sporozoites within the oocyst, each oocyst was categorized as sporulated or unsporulated (Conway and McKenzie, 2007). Three samples were drawn from each vaccine, and each sample was counted in accordance with species and sporulation status of the oocysts.

Vaccine Mixing

Each vaccine was mixed with either the gel diluent or water, at a dosage of 250 mL reconstituted gel per 1,000 doses or 240 mL of water per 1,000 doses. The gel powder was reconstituted according to manufacturer's protocol prior to mixing in the vaccine. To ensure even mixing of vaccine throughout the gel diluent, a handheld electric whisk was used to mix for 3 min. Vaccine in water diluent was mixed by stirring and inversion of the sealed vaccine container.

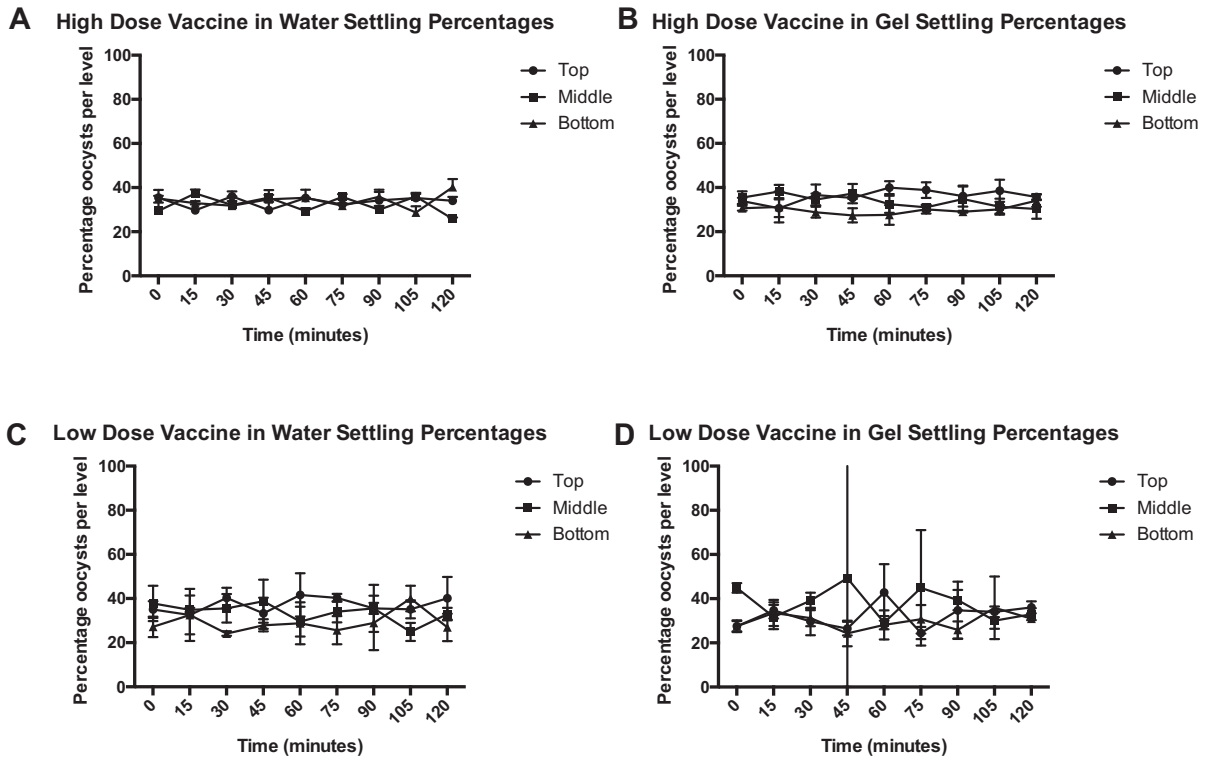

Figure 2. Determining oocyst settling over time, by a percent of oocysts present per level of the working stock of the vaccine. (A) High oocyst dose vaccine in water. (B) Low oocyst dose vaccine in water. (C) High oocyst dose vaccine in gel. (D) Low oocyst dose vaccine in gel.

Settling

To evaluate oocyst settling in water, 1 bottle of each vaccine was mixed in the appropriate amount of water and was continually aerated as recommended to maintain a uniform distribution of oocysts. Aeration was maintained utilizing a rubber hose with pinpoint holes attached to a low level continuous air source. Samples were drawn from 3 levels, the top, middle, and bottom, every 15 min for 2 h. To determine settling in gel, 1 bottle of each vaccine was mixed in the appropriate amount of reconstituted gel. Samples were drawn from the same 3 levels every 15 min for 2 h, and at 24 h. All samples were read using a McMaster's chamber. Three samples were read at each timepoint for each level for each vaccine and diluent combination.

Application Pattern

A commercial spray cabinet utilizing 2 angled spray nozzles or a gel drop bar cabinet was used to apply the vaccines in different diluents onto a sheet of plexiglass placed on top of a chick basket to determine the application pattern of each delivery method.

Oocysts Per Droplet

Each sheet of Plexiglass was divided into 6 even sections, the left, middle, and right section in the front and back of the plate. Droplets were collected from each section. For the vaccines applied via spray administration, droplets were categorized into 5 sizes based on recoverable volume: extra-small (<1 μl), small (~ 1 μl), medium (~ 5 μl), large (~ 10 μl), and extra-large (~ 15–30 μl). Five droplets of each obtainable size (small to extra-large) were acquired from each section and every oocyst present in the droplet was counted using salt floatation in a McMaster's chamber as described. For the gel vaccine application, only 1 size droplet was formed (~ 30 μl). Five droplets were randomly collected from each section of the plexiglass and every oocyst present in the droplet was counted using salt floatation in a McMaster's chamber. Three replicates of the application were obtained by passing 3 individual sheets of plexiglass through the application system for each vaccine and diluent combination, with droplets collected from each sheet of plexiglass.

Oocysts Per Dose

A dose of 24 mL per 100 chicks was used to calculate oocyst per dose present in the water diluent. A dose of 25 mL per 100 chicks was used as the dosage for oocysts present in the gel diluent. The total number of oocysts per bottle was divided by the number of doses present in the bottle to determine the number of oocysts per dose present in the bottle. For the dosage of the working stock and samples collected from the nozzle, the number of oocysts per milliliter was divided by the number of doses present per milliliter for each respective diluent.

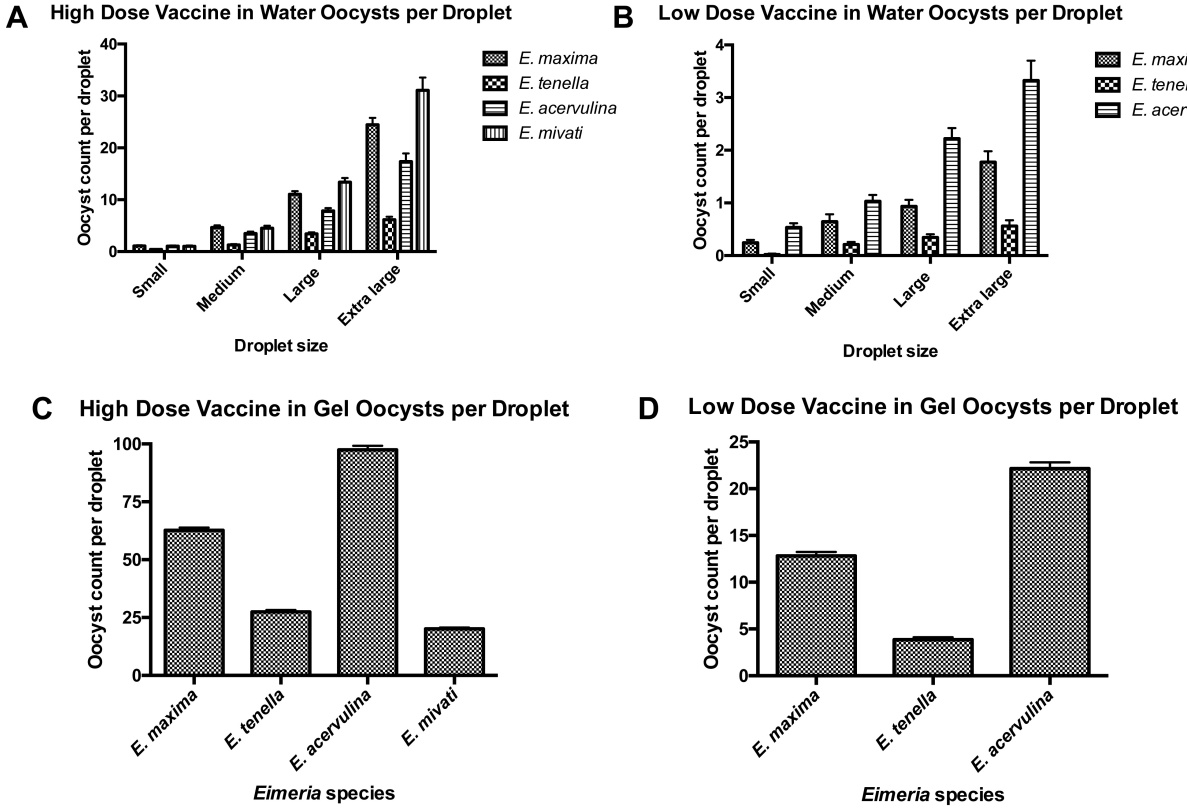

Figure 3. Number of oocysts present per droplet according to droplet size. (A) High oocyst dose vaccine in water. (B) Low oocyst dose vaccine in water. (C) High oocyst dose vaccine in gel. (D) Low oocyst dose vaccine in gel.

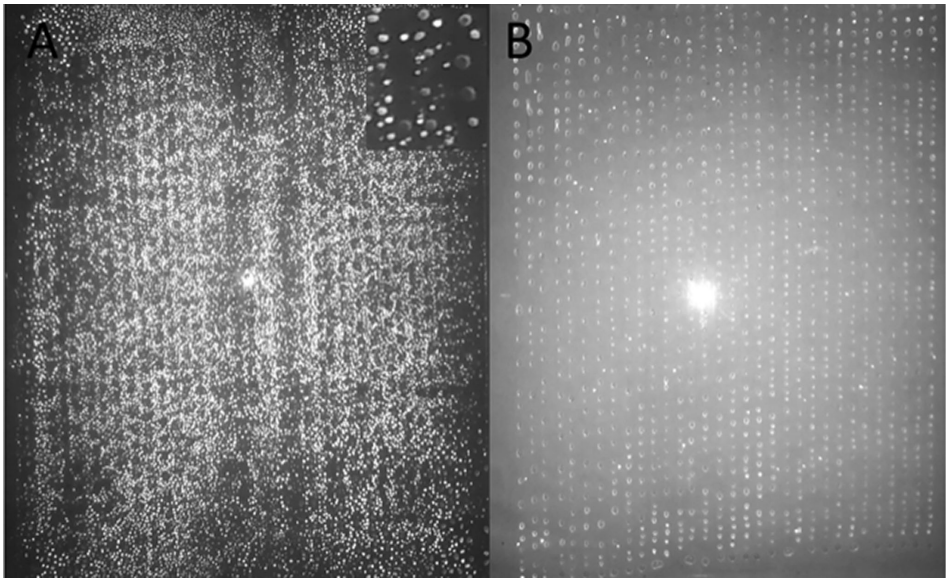

Figure 4. Image of plexiglass after being sprayed with each diluent (A) Spray applied with a 2-nozzle spray system. Insert magnification to show the variety of droplet size present. (B) Gel diluent applied with a gel drop bar

In order to determine the oocysts present per dose for the gel diluent, it was assumed that chicks ingested gel droplets for a dose of 250 μL per chick. To determine the number of oocysts present per dose for the water diluent, it was assumed that the chick would ingest an equal number of each size droplet to ingest a 240 μL dose per chick.

RESULTS AND DISCUSSION

Vaccine

Both vaccines showed a sporulation rate of >95%, indicating the majority of oocysts present could be capable of infection. The 3 species common to both vaccines were present in the same proportions with *E. acervulina*

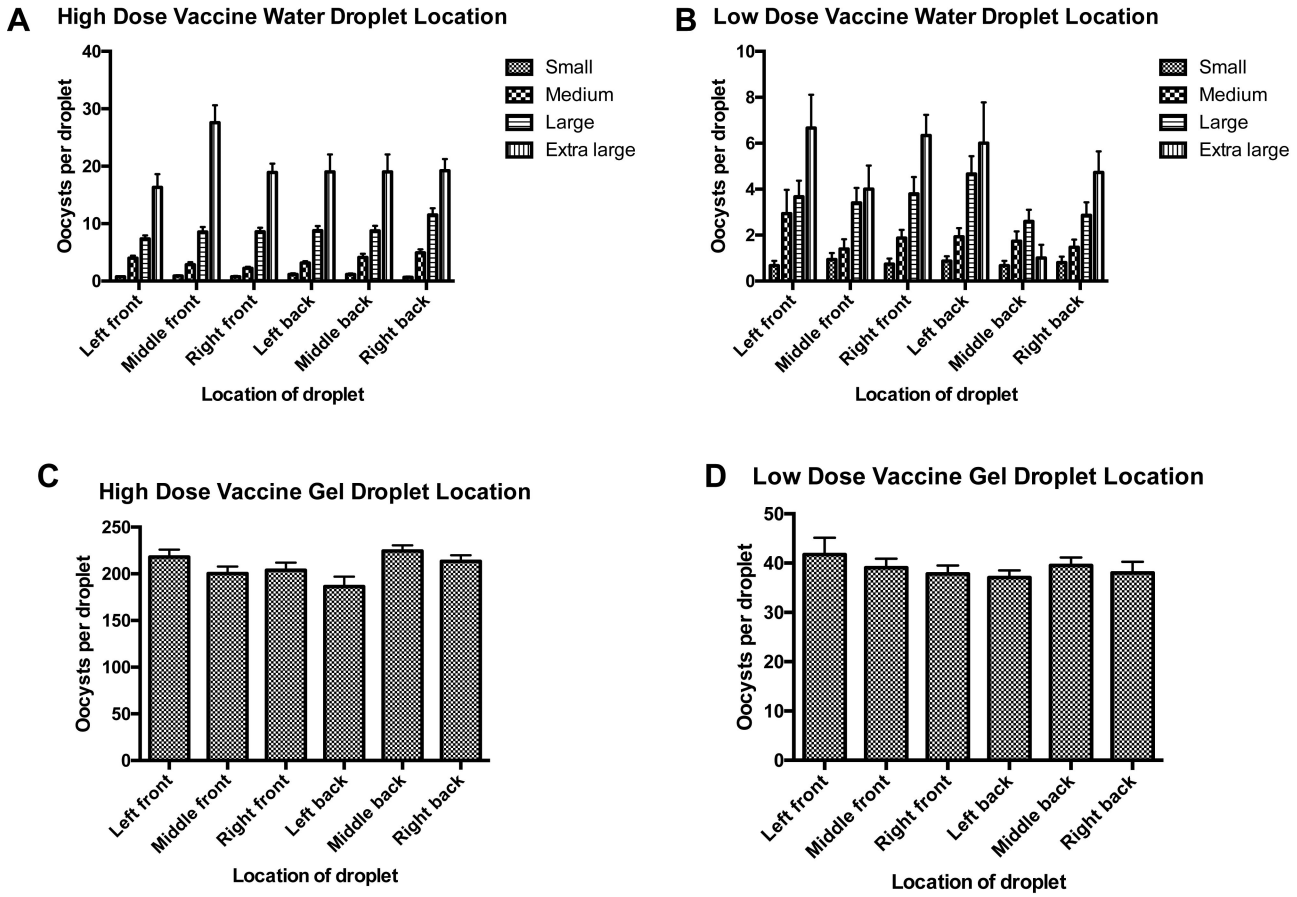

Figure 5. Number of oocysts present per droplet size according to the section of the droplet. (A) High oocyst dose vaccine in water. (B) Low oocyst dose vaccine in water. (C) High oocyst dose vaccine in gel. (D) Low oocyst dose vaccine in gel.

the highest, followed by *E. maxima* and *E. tenella.* The high oocyst dose vaccine contained ~1,630 oocysts per dose, nearly 6 times the number of oocysts in the low oocyst dose vaccine, which contained ~270 oocysts per dose (Figure 1).

Mixing

Although oocysts are extremely small, less than 30 μm, it is widely known that they settle when mixed in water unless a method to continuously agitate the solution is used (Landers, 1960; Long et al., 1976). As seen in this trial, when properly agitated, no settling occurred for either vaccine when mixed in a water diluent (Figure 2). Conversely, when using the gel product supplied for this trial, the oocysts did not settle and did not require any continual agitation (Danforth et al., 1997) (Figure 2). The gel diluent also claims it can be stored for 1 d after reconstitution and prior to use, so a sample was collected at 24 h post mixing to ensure that oocysts remained in suspension. Neither vaccine exhibited settling at this timepoint, indicating that a uniform application of vaccine in gel could still occur at 24 h. No application testing was performed at this timepoint; however, it is unknown how storage in the gel diluent would affect oocyst viability or infectivity. It should also be noted that diluting vaccine in gel diluent requires more rigorous mixing protocols and could be unevenly mixed if not done correctly. This would result in uneven vaccine distribution during application.

Spray Pattern

When vaccines in water-based diluent were sprayed on the plexiglass, the extra-large droplets were located primarily at the left and right edges (Figure 3A). There was an even distribution of the remaining sizes of droplets from side to side and front to back. When vaccines were applied in gel via gel drop bar, similar sized droplets were present across the entire sheet of plexiglass, with the 32 rows of droplets corresponding to the 32 "tips" present on the drop bar (Figure 3B).

Oocysts Per Droplet

The high oocyst dose vaccine applied in water diluent had oocysts present at each droplet size, and as the droplets increased in size, the number of oocysts also increased (Figure 4A). Oocyst counts per species remained in the same proportion as the vaccine bottle

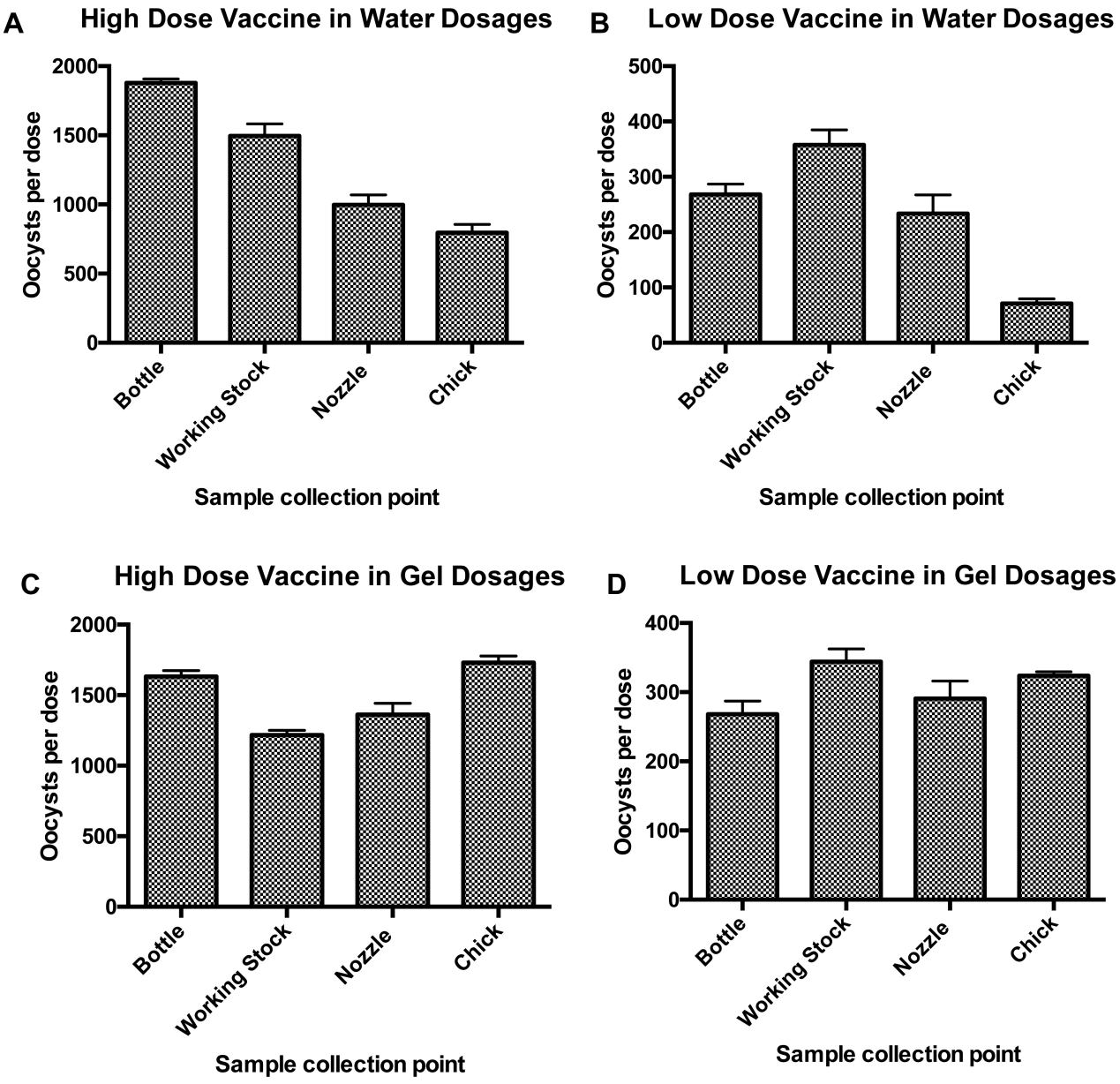

Figure 6. Number of oocysts present per dose, according to the sample collection point during vaccine application. (A) High oocyst dose vaccine in water. (B) Low oocyst dose vaccine in water. (C) High oocyst dose vaccine in gel. (D) Low oocyst dose vaccine in gel.

until the final time point, when *E. mivati* became the most common oocyst found. The low oocyst dose vaccine applied in a water diluent, when averaged across the 3 replicates, did not contain *E. tenella* in all droplets of any size (Figure 4B). *E. tenella* is the lowest proportion of species in the vaccine and is not in high enough concentration to be represented in all samples, when taking small quantities combined with the aerosolization of oocysts during delivery of the spray. The droplets also did not contain an average of at least 1 oocyst of the 2 other species until the large droplet size. The high oocyst dose vaccine applied in gel diluent had all 4 species present in the single droplet size, and in similar proportions as the vaccine bottle (Figure 4C). The low oocyst dose vaccine applied in gel diluent contained multiple oocysts of each species in each droplet (Figure 4D). As seen with the high oocyst dose vaccine, the low oocyst dose vaccine applied by either method maintained species proportionality with what was observed in the vaccine bottle. A common concern about mass application in hatcheries is uneven distribution across the chick basket (Chapman et al., 2002), though, in this trial, none of the vaccine and diluent combinations showed any difference in total number of oocysts present within each droplet size between the 6 sections on the plexiglass (Figure 5). Differences were seen when comparing the high and low oocyst dose vaccines for inclusion of every species in every size droplet, where droplets from the low oocyst dose vaccine applied by spray did not contain every species of oocyst in all droplets. This would result in uneven dosing of chicks if they do not consume at least 1 droplet of every size.

Dosages

Oocysts per dose were calculated at each collection point; vaccine directly from the bottle, vaccine mixed in the respective diluent, vaccine collected directly out of the nozzle or gel bar of the vaccination cabinet, and from the droplets applied to the plexiglass sheet in the chick basket (Figure 6). Comparing dosage numbers for spray application, there is a general decline in oocyst numbers for each collection point. This decrease is especially present between the oocyst counts in the working stock compared to oocyst counts from the spray nozzle, potentially due to the shearing effects of aerosolization from the nozzle. There is an additional decline in oocyst counts from the nozzle to what was collected on the plexiglass, which can be attributed to the smaller droplets that never made it to the plexiglass sheet due to external factors (air movement, natural fall rate of liquid droplets). This decline in vaccine reaching the chicks has also been noted in spray applied respiratory vaccines (Jordan, 2017).

The gel diluent and gel drop bar did not show this decline in oocysts reaching chick level, most likely due to the larger size and weight of the gel droplets, which are not influenced by external factors.

For coccidia vaccination to be successful, oocysts must reach the chicks in a uniform manner and this study shows that, regardless of vaccine or diluent, oocysts did reach the level of chicks in the chick basket. Oocysts from each vaccine were evenly distributed and remained in the proper proportion for each delivery method, indicating that each delivery system can effectively deliver vaccine without differentially affecting any particular coccidia species in the vaccines. Differences were seen in the effective dose of oocysts reaching chick level, with effects seen from application method and vaccine oocyst inclusion number. It remains to be seen how the difference in effective oocyst dose reaching chick level will influence vaccine oocyst ingestion by the chicks and, thereby, influence vaccine coverage and protection from challenge. In conclusion, these data demonstrate the similarities and differences between application characteristics of high and low oocyst dose coccidia vaccines when applied in water and gel diluents.

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